UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PA

AD-A143 870

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) USAFSAM-TR-84-296	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION USAF School of Aerospace Medicine 6b. OFFICE SYMBOL (If applicable) USAFSAM/RZP	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State and ZIP Code) Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas 78235	7b. ADDRESS (City, State and ZIP)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAF School of Aerospace Medicine 8b. OFFICE SYMBOL (If applicable) USAFSAM/RZP	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State and ZIP Code) Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas 78235	10. SOURCE OF FUNDING NOS. PROGRAM ELEMENT NO. 62202F; PROJECT NO. 7757; TASK NO. 01; WORK UNIT NO. 85

DTIC ELECTE AUG 3 1984 A

11. TITLE (Include Security Classification)
ANAMNESTIC CHEMILUMINESCENCE OF MURINE SPLEEN CELLS

12. PERSONAL AUTHOR(S)
Wong, Lenora S. and Kiel, Johnathan L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Final	FROM 2-15-83 TO 2-15-84	1984	6

16. SUPPLEMENTARY NOTATION
Reprint of a periodical article appearing in Immunological Communications, 13(3):285-290 (1984).

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB. GR.	Anamnestic immune response; Oxidative metabolism; Chemiluminescence
06	16		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The oxidative response of murine spleen cells to secondary exposure to antigen was determined by luminol (5-amino-2,3-dihydro-1,4pthalazinedione) amplified chemiluminescence, CL. BALB/cj and CBA/J mice were immunized with saline or an antigen solution of saline, luminol, and bovine serum albumin. Spleen cells were obtained from mice two and four days after immunization, and the CL response to in vitro antigenic exposure was measured for 35 minutes. At two days post-immunization, there was no difference in the CL of control and antigen-primed cells. By day four, the antigen-primed CL response differed significantly in both magnitude and time course from the primary antigen-stimulated response of the controls. This early development of differential CL response to antigenic challenge suggests a role for oxidative metabolic activity in the expression of the anamnestic immune response.

DTIC FILE COPY

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
Lenora S. Wong, 1Lt, USAF	512/536-3583	USAFSAM/RZP

DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE. UNCLASSIFIED

84 08 02 041

SECURITY CLASSIFICATION OF THIS PAGE

atch 4

IMMUNOLOGICAL COMMUNICATIONS, 13(3), 285-290 (1984)

ANAMNESTIC CHEMILUMINESCENCE OF MURINE SPLEEN CELLS

L.S. Wong and J.L. Kiel

Radiation Sciences Division
USAF School of Aerospace Medicine
Brooks Air Force Base, Texas 78235

Abstract

The oxidative response of murine spleen cells to secondary exposure to antigen was determined by luminol (5-amino-2,3-dihydro-1,4-pthalazinedione) amplified chemiluminescence, CL. BALB/cj and CBA/J mice were immunized with saline or an antigen solution of saline, luminol, and bovine serum albumin. Spleen cells were obtained from mice two and four days after immunization, and the CL response to in vitro antigenic exposure was measured for 35 minutes. At two days post-immunization, there was no difference in the CL of control and antigen-primed cells. By day four, the antigen-primed CL response differed significantly in both magnitude and time course from the primary antigen-stimulated response of the controls. This early development of differential CL response to antigenic challenge suggests a role for oxidative metabolic activity in the expression of the anamnestic immune response.

Introduction

The production of peroxides and other active oxygen species is one of the earliest measurable signals of immunogenic stimulation (1,2). Alterations in oxidative activity have long been correlated with the phagocytic and bactericidal activity of macrophages and polymorphonuclear neutrophils, although CL has also been associated with the activation of lymphocytes (2) and thymocytes (3). Increased peroxidase activity of mouse splenic lymphocytes (4,5) and human peripheral lymphocytes (6) in response to antigenic exposure in vitro has been reported. In vivo exposure to antigen also results in enhanced activity of the enzyme (1). That oxidative metabolic activity plays an early role in immune cell function is apparent. This paper reports the rela-

Copyright © 1984 by Marcel Dekker, Inc.

0090-0877/84/1303-0285$3.50/0

tionship of murine splenocyte *in vitro* CL with previous *in vivo* exposure to antigen.

Materials and Methods

Reagents. Hank's Balanced Salt Solution (HBSS) buffered with 20 mM HEPES, pH 7.4, was the medium for all experiments. The luminol (5-amino-2, 3-dihydro-1,4-phthalazinedione) mixture for immunizations was prepared by suspending 3 mg/ml luminol (Baker Chemical Co., Phillipsburg, NJ) and dissolving 3 mg/ml Bovine Serum Albumin, BSA (Sigma Chemical Co., St. Louis, MO) into phosphate buffered saline, PBS, pH 7.4. The luminol indicator for the CL assay was a 1:5 dilution of a freshly prepared solution of 1 mg/ml luminol, 1 mg/ml BSA in PBS, pH 6.9. The luminol mixtures were prepared at room temperature, filtered (Gelman Acrodisc, 0.2 um) to remove undissolved luminol and stored at 4°C in the dark until use.

Animals. Male CBA/J or BALB/cj mice (Jackson Laboratories, Bar Harbor, ME), 25-30 g., were maintained on a 12/12 light/dark cycle with food and water *ad libitum*. Control animals were immunized with 0.5 ml saline (subcutaneous or intraperitoneal) and experimental animals received 0.2 ml luminol-BSA antigen (subcutaneous). CL was assayed 2 or 4 days after immunization.

Spleen Cell Preparation. Mice were killed by rapid cervical dislocation and their spleens removed. The spleens were gently teased in room temperature HBSS (20 mg spleen/ml HBSS). The suspension was centrifuged at 6 x g for 3 minutes to remove residual tissue. Cell viabilities were determined by exclusion of trypan blue dye, and the cell concentration was adjusted to $8\text{-}12 \times 10^3$ viable cells/mm^3. Differential counts made of stained fixed slides showed suspensions to be 94-96% lymphocytes. One ml samples in dark-adapted glass scintillation vials were incubated for 25 min at 37°C in a shaker water bath.

Assay of Chemiluminescence. After the incubation period, 100 ul of the luminol-antigen mixture was added to each vial. CL was measured before, at, and every 5 minutes after the addition of luminol, on a Beckman model LS230 liquid scintillation counter in the "out of coincidence" mode (gain 350, preset 0.2 min, window 0-100). Cells were maintained at 37°C between CL readings.

Statistical Analysis. A repeated measures analysis of variance was used to determine differences in the time dependent variation of the CL response between groups. The average CL value for each group, determined from the total of all CL measurements between T=5-35 min, was compared using Duncan's multiple range test.

Results

The CL response of murine splenocytes to *in vitro* primary and secondary exposure to antigen is illustrated in Figure 1. As no differences were seen in the responses of the two strains of mice, the data were pooled. At two days post-immunization, the oxidative activity was higher, although not sig-

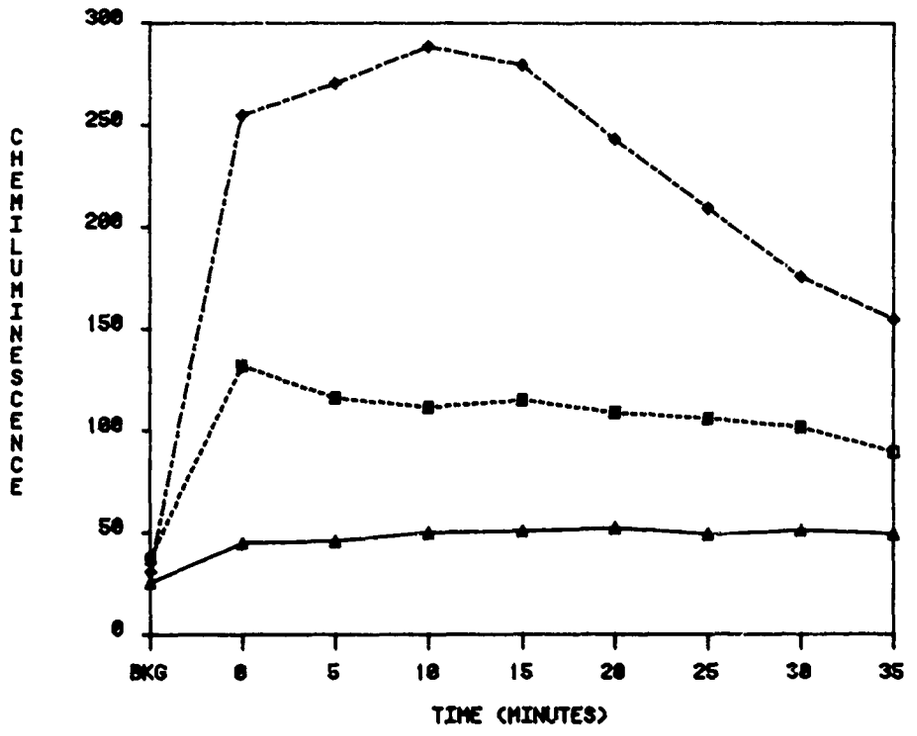

FIGURE 1.

Chemiluminescent response of murine spleen cells to primary and secondary antigenic exposure. The oxidative response to antigen was determined for unprimed cells (Δ-Δ) and cells taken from mice two days (□-□) and four days (◇-◇) after immunization. Luminol amplified CL is expressed in 10^3 cpm. Points represent the mean of 3 or 4 animals.

nificantly different, from that of unprimed cells. By four days, however, the response was greatly enhanced; the time course of CL differed at the P=0.0001 level, and mean overall CL was significantly greater (Table I) than for either control or two-day groups.

Discussion

The CL responses shown here demonstrate the enhancement of antigen stimulated oxidative metabolic activity which results after prior *in vivo* immunologic exposure. Whether the change in oxidative activity, as measured here by CL, is a cause or by-product of the anamnestic response is unclear; however, it is certain that alterations in oxidative activity play a role in the very early immune response to secondary antigenic challenge. Both the production of peroxides *per se* and the resulting peroxidation of membrane surface macromolecules may be involved in the anamnestic immune response.

The increase in CL activity seen at 4-days post-immunization supports previous reports of enhanced oxidative capacity in activated lymphocytes. CL and H_2O_2 production have been described in antigen and mitogen stimulated

TABLE I

Total and average chemiluminescent responses of murine splenocytes following *in vivo* exposure to antigen

Group	Total CL[a] (cpm x 10^3)	Average CL[b] (cpm x 10^3)
Control (4)[c]	169.4 ± 68.7[d]	24.2 ± 25.9 (28)[e]
2 Day (3)	484.7 ± 155.6	69.2 ± 58.8 (21)
4 Day (3)	1405.8 ± 439.8	200.8 ± 166.2[f] (21)

[a]cpm above background from T=5-35 min
[b]Mean of all CL measurements from T=5-35 min
[c]Number of animals
[d]Mean ± S.D.
[e]Number of measurements
[f]Mean differs from both control and 2 Day; $p<.01$, Duncan's multiple range test

mouse (3) and rat (2) lymphocytes. Paul et al (4) reported peroxidase activity in murine splenocytes in the presence of particulate antigens. The splenic peroxidase (SPO) increased in activity after animals were immunized with soluble or particulate antigens, with maximal enzyme activity preceeding the appearance of antibody producing cells. In the presence of H_2O_2, this SPO is bactericidal *in vitro* (5) and also mediates the deamination of amino acids and production of CO_2 and aldehydes (7).

Peroxidation of the cell surface resulting from the increased CL activity seen here may serve in lymphocyte activation and immuno-amplification. Cell surface modification by sodium periodate or neuraminidase and galactose oxidase has been shown to induce blastogenesis and nonspecific cytotoxicity in normal mouse lymphocytes (8,9,10) and antigen specific cytotoxicity in "memory" cells which had been previously exposed to antigen *in vivo* or *in vitro* (9,10). Additionally, $NaIO_4$ or NAGO modified cells can indirectly stimulate secondary cytotoxicity from syngeneic cells in mixed lymphocyte culture (11). In rat lymphocytes, indirect NAGO stimulation selectively activates cytotoxicity rather than blastogenicity (12). The stimulatory effects of membrane peroxidation are dependent upon the presence of induced free surface aldehydes (8,11) which may crosslink membrane macromolecules (via a Schiff base) on the same cell or between cells (8).

It is possible, then, that initial antigenic exposure gives rise to a population of "memory lymphocytes" containing enhanced SPO activity, which may be localized at the cell membrane (1). Upon secondary antigenic challenge, these cells produce or induce the ananmestic oxidative response we report here; with the generated H_2O_2 (in the presence of SPO) inducing free aldehydes (7) on cell surfaces. Direct peroxidation of nearby normal lymphocytes results in blastogenesis and some nonspecific cytotoxicity (8,10); peroxidation of memory lymphocytes results in antigen specific cytotoxicity (9,10) and indirect stimulation of normal, surrounding lymphocytes selectively stimulates cytotoxicity (12). In such a way, enhanced oxidative response to secondary antigenic challenge and the resulting peroxidation of cell surface macromolecules may orchestrate the amplification of specific and nonspecific anamnestic immune activity.

Acknowledgements

We thank Mr. Don Cosgrove for his statistical analysis of the data, and Ms. Jacque West for her support in the preparation of the manuscript.

References

1. R.R. Strauss, H. Friedmann, L. Mills, and G. Zayon, Infect. Imm., 15, pp. 197-203 (1977).

2. K. Wrogemann, M.J. Weidemann, B.A. Peskar, H. Staudinger, E.T. Rietschel, and H. Fisher, Eur. J. Immunol., 8, pp. 749-752 (1978).

3. B. Descamps-Latscha, R.M. Golub, A.T. Nguyen, and M-N. Feuillet-Fieux, J. Immun., 131, pp. 2500-2507 (1983).

4. B.B. Paul, R.R. Strauss, A.A. Jacobs, and A.J. Sbarra, Infect. Imm., 1, pp. 338-344 (1970).

5. R.R. Strauss, B.B. Paul, A.A. Jacobs, and A.J. Sbarra, Infect. Imm., 5, pp. 114-119 (1972).

6. B.B. Paul, J. Reticuloendothel. Soc., 25, pp. 49-60 (1979).

7. R.R. Strauss, B.B. Paul, A.A. Jacobs, and A.J. Sbarra, Infect. Imm. 5, pp.120-126 (1972).

8. A. Novogrodsky, J. Immun., 114, pp. 1089-1093 (1975).

9. R.C. Kuppers and C.S. Henney, J. Immun., 119, pp. 2163-2171 (1977).

10. J.L. Maryanski, J-C. Cerottini, and K.T. Brunner, J. Immun., 122, pp. 342-349 (1979).

11. E.A. Grimm and B. Bonavida, J. Immun., 123, pp. 2026-2033 (1979).

12. C.F. Beyer, and W.E. Bowers, J. Immun., 121, pp. 1790-1798 (1978).

Accession For
NTIS CRA&I
DTIC TAB
Unannounced
Justification
Distribution /
Availability Codes
Dist | Avail and/or Special
A-1 | 20

DTIC COPY INSPECTED